CYTOLOGICAL STUDIES IN THE MYRTACEAE.

I. MICROSPOROGENESIS IN SEVERAL GENERA OF THE TRIBE LEPTOSPERMOIDEAE.

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(Plate xi; twenty-two Text-figures.)

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Information on the cytology of the Myrtaceae is very meagre. This may be due partly to the difficulties inherent in the material, and partly to the fact that only in Australia does the family constitute a dominant and important section of the indigenous flora.

McAulay and Cruickshank (1937) have determined the chromosome numbers in certain Tasmanian species of *Eucalyptus*, and have outlined the male meiotic cycle in these species. To the author's knowledge, however, no other work has been published on the cytology of the family. Systematists such as Blakeley (1934) and Maiden (1909–1929) have stressed the importance of hybridization in *Eucalyptus*, and have suggested that many of the recognized species are of hybrid origin. Brett (1937) has classified Tasmanian Eucalypts into several groups according to the status of the species—simple and compound Linnean species, polymorphic species, and first generation hybrids. It is doubtful whether the latter deserve specific rank. Lawson (1930) has discussed the origin of the Australian flora from the view-point of the high percentage of endemism which occurs, and concludes that many features, particularly the high degree of pollen and seed sterility found in the Myrtaceae, Proteaceae and other dominant families, indicate the extensive occurrence of hybridism in its development. If such hybridism has occurred, evidences of it should be found in the chromosomes and in irregularities of behaviour at meiosis.

Materials and Methods.

Table 1 presents details of the species which have been studied, the nomenclature adopted being that of Blakeley (1934) for *Eucalyptus*, and of Maiden and Betche (1916) for the other genera. All the species belong to the tribe Leptospermoideae. The three species of *Angophora* are the most common representatives of that genus on the sandstone country in the vicinity of Sydney. As they often occur intermixed, and as their flowering periods overlap, some hybridization should be expected. Several suspected hybrid forms have been seen, besides the form which has been examined which has the general characters of *A. cordifolia*, but differs in the less glandular buds, and the shortly but distinctly petiolate leaves. Amongst the representatives of *Eucalyptus*, members of several of the main series are included. *E. ficifolia* and *E. ficifolia* var. *Guilfoylei* (series Corymbosae-peltatae) are Western Australian species which have been extensively planted around Sydney. The latter is reputed to be a garden hybrid between *E. ficifolia* and the closely related *E. calophylla*, and shows considerable variation particularly in flower colour. *E. gummifera* (Syn. *E. corymbosa*) (Corymbosae-peltatae) is an eastern species which, at least in the district studied, is an isolated member of its series. It is a relatively uniform species. *E. incrassata* (Dumosae) belongs to the Western Australian mallees.

TABLE 1.
Details of the Material.

Species.	Locality.	Original Source.	No. of Plants Examined.	Availability of Material.
Angophora intermedia DC. ..	Gordon, N.S.W.	Wild.	4	Oct.-Nov.
A. lanceolata Cav.	Gordon, N.S.W.	Wild.	2	Oct.-Nov.
A. cordifolia Cav.	Gordon, N.S.W.	Wild.	5	Oct.-Nov.
A. cordifolia X.	Gordon, N.S.W.	Wild.	1	October.
Eucalyptus gummifera Sm.	Gordon, N.S.W.	Wild.	3	January.
E. ficifolia F. v. M.	Gordon, N.S.W.	Cultivated W. Aust. species. Seed source unknown.	1	December.
E. ficifolia var. *Guilfoylei* Bail.	Gordon, N.S.W.	Cultivated W. Aust. species. Seed source unknown.	2	Nov.-Dec.
E. incrassata Labill. ..	Botanic Gardens, Sydney.	Cultivated W. Aust. species.	1	January.
E. haemastoma Sm.	Gordon, N.S.W.	Wild.	2	Dec.-Jan.
E. sideroxylon (A. Cunn.) Benth.	Lindfield, N.S.W.	Avenue trees. Seed source unknown.	4	Jan.-Mar.
E. paniculata Sm.	Gordon, N.S.W.	Wild.	1	March.
E. dives Sch. (normal) ..	Lindfield, N.S.W.	Cultivated 4 yr. old tree. Seed from Braidwood, N.S.W.	1	December.
E. dives var. A.	Lindfield, N.S.W.	Cultivated 3 yr. old tree. Seed from Officer, Victoria.	1	December.
E. dives var. C.	Lindfield, N.S.W.	Cultivated 3 yr. old tree. Seed from Tumbarumba, N.S.W.	1	December.
Tristania conferta R.Br. ..	Gordon, N.S.W.	Cultivated avenue trees. Seed source unknown.	3	November.
Backhousia citriodora F. v. M.	Gordon, N.S.W.	Cultivated garden tree. Seed source unknown.	1	November
Leptospermum citratum Chall., Cheel and Penf.	Lindfield, N.S.W.	Cultivated 4 yr. old tree. Seed from Copmanhurst, N.S.W.	1	December.
L. citratum	Lindfield, N.S.W.	Cultivated 3 yr. old tree. Seed from Brebi I., Queensland.	1	December.
Callistemon linearis DC. ..	Gordon, N.S.W.	Probably cultivated. Seed source unknown.	2	Mar.-Apr.
C. lanceolatus DC.	Gordon, N.S.W.	Cultivated. Seed source unknown.	1	Mar.-Apr.

E. haemastoma (Psathryoxylon) occurs together with several closely related species on the sandstone country of coastal New South Wales. *E. sideroxylon* var. *rosea* and *E. paniculata* (Siderophloiae) are "Ironbarks", and the former, which has been extensively planted for ornamental purposes, shows very conspicuous variation in flower colour, leaf shape, glaucousness and other characters, and is possibly of hybrid origin. *E. dives* (Piperitales) is an eastern tableland species which Penfold and Morrison (1927) have shown to occur in several distinct varieties, differing mainly in the nature of their essential oils. A great deal of hybridization seems to have taken place in this series, and species determination is often very difficult.

Tristania conferta is a uniform species, representative of a small genus of brush forest trees, which also has been extensively planted for street purposes. *Backhousia citriodora* and *Leptospermum citratum* are species of restricted distribution, producing essential oils of commercial value. The latter is closely related to, and possibly recently derived from, *L. flavescens*. Recently Penfold *et al.* (1942) have reported the occurrence of two forms of *L. citratum* differing markedly in oil characteristics.

Callistemon lanceolatus and *C. linearis* are "Bottlebrushes", several species of which are cultivated as garden shrubs, and which are known to be interfertile.

The studies herein reported were made on meiotic division in the microspore mother cells. The study of somatic mitosis in root tips, leaf buds, and young anther tissue offers difficulties owing to the small size of the somatic nuclei and the crowding of the

chromosomes, but in a few cases confirmation of chromosome numbers from such material has been made.

In some preliminary work on microsporogenesis, material was fixed in Flemming and Navashin type fixatives, and sectioned by a paraffin technique, but a modified acetocarmine smear method was found to give equivalent results and has been generally adopted. Direct acetocarmine smears according to the method of Belling (1930) gave unsatisfactory results, apparently owing to the presence of tannins or resinous substances, which appeared to cause a heavy precipitation of the stain in the cytoplasm, and also to the presence of numerous minute oil globules which obscured details. Following Thomas (1940), premordanting was combined with prefixation in Farmer's acetic alcohol by saturating the glacial acetic acid used with carmine and adding ferric acetate until this solution assumed a rich wine colour. It was found necessary, however, to remove gummy and resinous substances by first fixing the material in ordinary fixative before using the premordant. In changing the material from the ordinary fixative to the premordant, brief immersion in a mixture of absolute alcohol and hydrochloric acid (2:1) was found to be an advantage, aiding the removal of resinous substances and facilitating later smearing.

The schedule finally adopted is set out briefly hereunder:

1. The anther masses are cut out, as illustrated in Figure 1, and are immediately dropped in ordinary Farmer's acetic alcohol (1:3) for 2 to 3 hours. If the fixative becomes heavily coloured with extracted resinous substances, it should be changed after 30 minutes.
2. Transfer for 5 to 10 minutes to a mixture of absolute alcohol and hydrochloric acid (2:1).
3. Transfer to carmine-acetic-alcohol, made up according to Thomas' method. The buds should remain in this fluid for at least 2 hours, but preferably overnight.
4. Smear a few anthers in iron acetocarmine, cover, warm, and seal. The optimum amount of iron in the acetocarmine must be determined for each species and each sample of carmine. Usually less iron than that indicated in Belling's formula is required.

Material fixed at 10 a.m. has given good metaphase I and later stages, but good early prophase I stages are not obtained owing to the severity of the fixative. In buds at a suitable stage of development, all meiotic stages may be obtained. The reduction division first occurs in the anthers on the outer filaments, or in the anther mass as they are folded in the bud—at (*a*) Figure 1—and follows progressively towards the centre of the flower. When anthers at (*a*), Figure 1, show fully-formed microspore nuclei, anthers at (*b*) may show second division stages, and anthers at (*c*) may be in stages as early as prophase I. The divisions do not occur uniformly in the one anther, but show a gradation from base to apex in each locule.

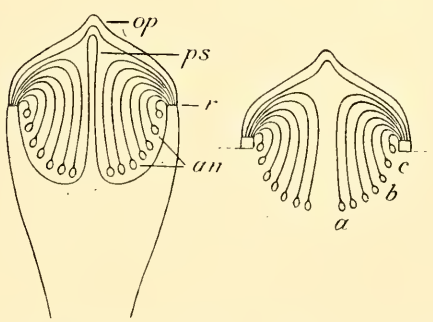


Fig. 1.—Diagrammatic longitudinal sections of buds of *E. gummifera* to illustrate method of cutting, and incidence of meiosis. Approx. × 6. *op.*, operculum; *r.*, rim; *ps.*, pistil; *an.*, anthers. See also text.

Drawings were made with a 2 mm. apochromatic objective and a 20X compensating eye-piece, using an Abbe camera lucida, giving magnification at bench level of 3,350. Photographs are at an approximate magnification of 2,000.

The Meiotic Cycle.

The general behaviour at meiosis in the microscope mother cells appears very similar in all the genera studied. After the last premeiotic mitosis the cells undergo a period of development. The considerable increase in the size of the nuclei, which occurs during this period and which continues during early prophase I, is possibly simultaneous with an increase in their chromatin content. The degree of increase in size is indicated by the average diameter of the nuclei, 5–7μ for resting nuclei and 10–12μ for nuclei at late prophase I.

The nuclei are of the vesicular type, described by Manton (1935). A single large nucleolus may be placed either centrally, or more often markedly to one side (Pl. xi, figs. 1, 13).

A detailed study of prophase I development has not been made, owing to the unsuitability of the technique adopted, but this development appears normal. At pachytene and diplotene, the occurrence of chiasmata has been observed, and in *E. gummifera* chiasmata may vary from 1 to 3 per chromosome pair. Throughout prophase I the chromosomes contract, until at diakinesis they have become closely paired short-oval to spherical bodies arranged near the surface of the nucleus (Pl. xi, fig. 14). By this time the chiasmata appear to be completely terminalized.

Transition to metaphase I is normal, and this stage has proved most satisfactory for the purpose of chromosome counts. In all the species studied the haploid number of $n = 11$ has been found, with no evidence of polyploidy, although some secondary association of the chromosomes is general. The chromosomes show only slight differences in size, measuring 0·9–1·3μ in length, and only rarely is it possible to identify a particular bivalent.

Separation of the chromosomes at anaphase I is normal, with terminalized chiasmata at one or both ends. The occasional failure of terminalization with resultant lagging or non-disjunction is discussed later. In the transition from anaphase I to telophase I the chromosomes become bunched into a mass (Pl. xi, fig. 6) before opening up to form the interphase nuclei.

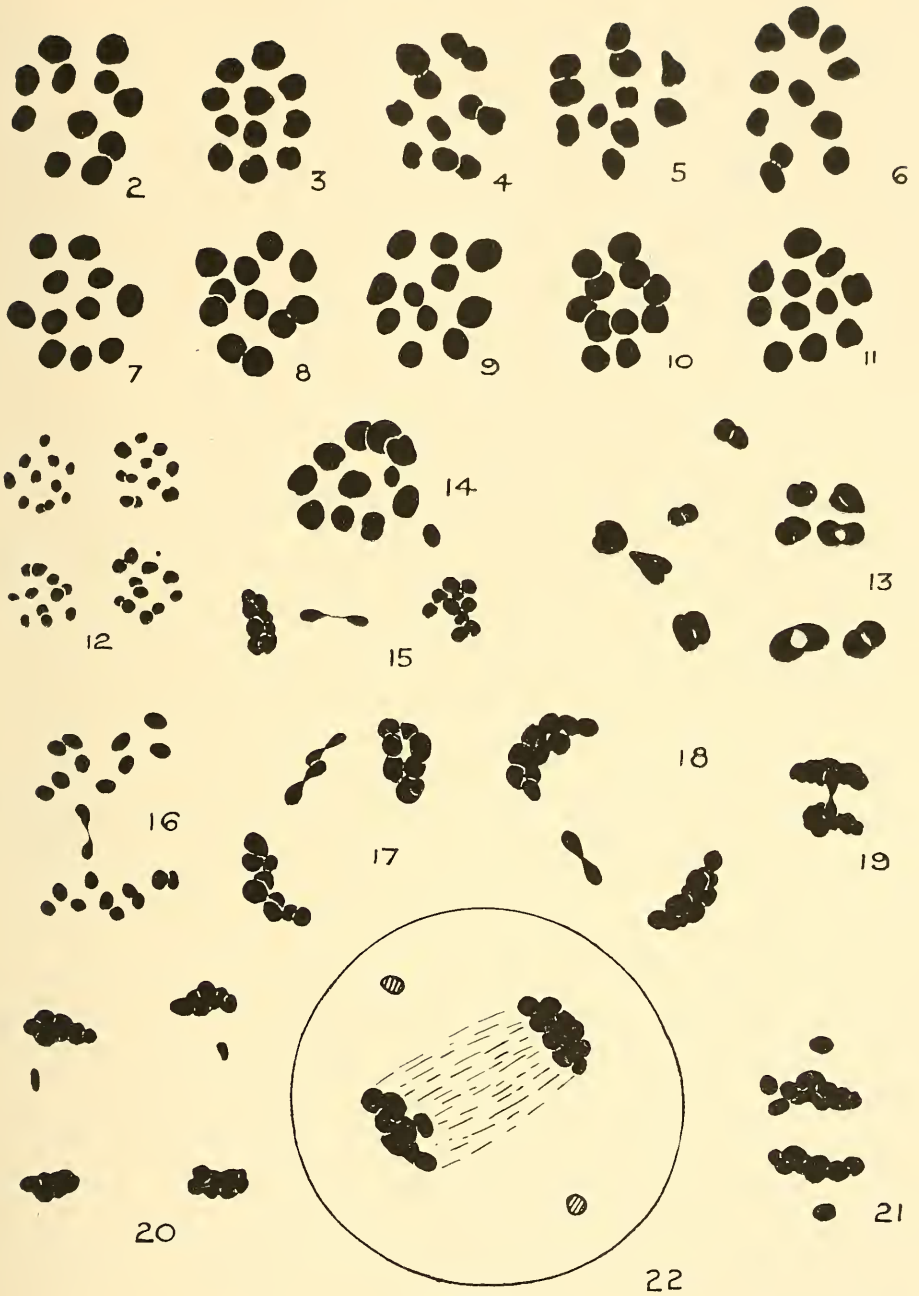
The chromosomes do not appear to lose their identity during the interphase, which is apparently of short duration, and in the subsequent division the chromosomes do not regain their full mitotic length.

Particularly during anaphase I in *L. citratum* and *B. citriodora*, two peculiar bodies lying in the cytoplasm on either side of the spindle (Fig. 22) have been observed, and it is possible that these occur also in the other genera. The origin and behaviour of these bodies have not yet been studied, but they are possibly the structures described by Castetter (1925) in *Melilotus* and by Beard (1937) in *Echinocereus*.

At the commencement of prophase II the chromosomes are more or less evenly distributed over the surface of the two nuclei, but they soon come to lie on the surface of each nucleus internal to the cell, giving the "saucer" stage described by McAulay and Cruickshank (1937). This behaviour is most conspicuous in *Eucalyptus* and *Angophora*, is less evident in *Tristania*, and is not apparent in *Leptospermum* or *Backhousia*. The double nature of the chromosomes is now evident, and the attachments appear median or submedian. Following this stage, the membranes and nucleoli are absorbed and at metaphase II the chromosomes become arranged on their respective spindles, which may lie in the same plane or at right angles to one another. Telophase II and the reorganization of the microspore nuclei are normal. Cytokinesis does not occur until after the reorganization of the microspore nuclei.

Special Features in Meiosis.

Most important among irregularities in chromosome behaviour during microsporogenesis are secondary pairing and the occurrence of non-disjunction and lagging at anaphase I, apparently due to the failure of terminalization of chiasmata. The occurrence of a proportion of aborted pollen in some of the species may be due to this fact.



Figs. 2-22.

All drawings have been made with the camera lucida from acetocarmine preparations. Figure 12 has been reduced to a magnification of 1,700 diameters. The others are all at 3350 ×.

Figs. 2-11.—Chromosome complements at metaphase I. 2.—*Eucalyptus ficifolia* var. *Guilfoylei*. 3.—*E. gummifera*. 4.—*E. haemastoma*. 5.—*E. dives* var. A. 6.—*Callistemon lanceolatus*. 7.—*Tristania conferta*. 8.—*Angophora lanceolata*. 9.—*A. cordifolia*. 10.—*Backhousia citriodora*. 11.—*Leptospermum citratum*. Fig. 12.—Secondary pairing in *E. gummifera*. Fig. 13.—Chromosomes of *E. dives* spread by pressure on the slide. Fig. 14.—Failure of pairing at metaphase I in *L. citratum*. Figs. 15-18.—Non-disjunction and lagging at anaphase I. 15.—*E. ficifolia* var. *Guilfoylei*. 16.—*A. cordifolia*. 17 and 18.—*L. citratum*. Fig. 19.—Chromosome bridge at anaphase II in *B. citriodora*. Fig. 20.—Simultaneous lagging at anaphase II in *B. citriodora*. Fig. 21.—Precocious separation of chromosomes at anaphase I in *L. citratum*. Fig. 22.—Cytoplasmic bodies in *B. citriodora*.

There is also a rare failure of pairing between the individual chromosomes of a bivalent pair, with consequent precocious separation at anaphase I.

Secondary Pairing.

The occurrence of secondary association has already been mentioned. This association reaches a maximum of four pairs of bivalents, but is variable in the different species, and in some the occurrence of triple groupings and irregular associations makes the phenomenon more difficult of interpretation. The degree of secondary pairing in *E. gummifera* is given in Table 2. This species shows a minimum of irregular association and constancy in meiotic behaviour. In *E. dives* and *E. haemastoma*, and in *Angophora* spp., *Backhousia* and *L. citratum* irregular associations are common, and it is noteworthy that in all of these species hybridization with related and associated species has been possible, and is suspected. *Tristania conferta* shows a constancy equivalent to that of *E. gummifera*.

TABLE 2.

Frequency of Secondary Pairing in E. gummifera.

Number of Secondary Pairs.	Observed Frequency.
0	3
1	5
2	16
3	17
4	15
Total	56

This general occurrence of secondary pairing suggests the derivation of the normal haploid chromosome complement from an original basic set of seven, but it would appear that such derivation must be primitive, perhaps to the evolution of the whole family. Cytological examination of the most primitive genera in the family and of allied families is desirable.

Non-disjunction and Allied Irregularities.

Apparent failure of terminalization occurs with a variable frequency in the several species, causing a variable frequency of such irregularities as non-disjunction and lagging at anaphase I. In Table 3 this frequency is shown, whilst in two species of *Angophora* and in *Tristania conferta* no cases have been observed, but may occur rarely. Since the observations were made on material fixed during intervals of several months, under widely varying weather conditions, the figures are qualitative only, as various authors, such as Sax (1935) and Anderson and Sax (1936) have shown that temperature may affect chiasma formation and its dependent phenomena.

In *E. dives* lagging occasionally leads to the exclusion of chromosomes from the interphase nuclei, and such deficient nuclei probably degenerate. In *L. citratum* the occurrence of lagging is most frequent, and often two bivalents in the one spindle show non-disjunction. This is consistent with the probably recent origin of the species, as judged by its occurrence and distribution in the field.

Unexplained lagging at anaphase II occurs in *L. citratum* and *B. citriodora*, and such lagging is often simultaneous in the two spindles (Fig. 20). Insufficient data have been obtained to indicate its frequency or cause.

Failure of Metaphase I Pairing.

This is a rare occurrence and has only been observed in *Leptospermum* and *Backhousia*. It would lead to precocious separation at anaphase I, which has also been observed in these two species. It may be purely accidental, or due to a certain lack of homology between the chromosomes concerned.

TABLE 3.

Frequency of Non-disjunction and Lagging at Anaphase I.

Species.	Number of Observations.	Number of Observed Irregularities.
Angophora cordifolia Cav.	22	3
Eucalyptus ficifolia var. *Guilfoylei* Bail.	43	1
Eucalyptus gummifera Sm.	385	1
Eucalyptus dives var. A.	50	5
Backhousia citriodora F. v. M. ..	20	3
Leptospermum citratum Chall., Cheel and Penf.	72	11

SUMMARY AND CONCLUSIONS.

1. A rapid method for the study of meiosis, adapted to the Australian Myrtaceae, has been developed. The method is not suited to the study of early prophase I stages, but gives good results for other stages, and is particularly suited to chromosome counts.

2. Using this method, a study has been made on material from thirty-eight plants, representing sixteen species and six genera of the tribe Leptospermoideae of the Myrtaceae. In the genus *Eucalyptus*, eight species, representing five series, have been examined.

3. The haploid chromosome set in all species is eleven. The chromosomes are small and not well suited to the study of chromosome structure.

4. The male meiotic cycle described by McAulay and Cruickshank for *Eucalyptus* has been confirmed and has been shown to apply to the other genera with only slight modifications.

5. The occurrence of secondary pairing between bivalents at metaphase I suggests the derivation of the haploid chromosome set from a basic set of seven, but this derivation is almost certainly primitive and requires additional evidence. Secondary association becomes irregular in those species in which hybridization is suspected.

6. Chiasmata have been observed to a maximum of three per bivalent, and terminalization is normally complete. The occasional apparent failure of this terminalization, leading to non-disjunction and other irregularities, is possibly due to chromosome irregularities which have not yet been fully stabilized. The greatest degree of irregularity is found in those species, such as *Eucalyptus dives*, *Leptospermum citratum* and *Backhousia citriodora*, which on other grounds are presumably of the most recent origin, or which show the greatest degree of suspected hybridization. Well-established species showing little natural variation, such as *Eucalyptus gummifera* and *Tristania conferta*, are most stable.

7. Pairing of chromosomes at metaphase I rarely fails, and then only in those species showing a maximum of other irregularities. It may be due to a certain lack of homology leading to a reduction in chiasma frequency.

EXPLANATION OF PLATE XI.

All photographs are from acetocarmine preparations, and are at a magnification of approximately 2,000 diameters.

1.—Prophase, *E. ficifolia*. 2.—*E. haemastoma*. 3.—Metaphase I, *E. haemastoma*, showing secondary association. 4.—Metaphase I, *E. gummifera*. 5.—Metaphase I, *E. dives* var. A. 6.—Telophase I, *E. haemastoma*. The chromosomes are closely bunched prior to opening out into the interphase nuclei. 7.—Metaphase II, *E. dives*. 8.—Telophase II, *E. haemastoma*. The eleven chromosomes can just be made out in one group. 9.—Metaphase I, *A. cordifolia*. 10.—Metaphase I in side view, *A. cordifolia*. The overlapping of the chromosomes makes their examination in this position impossible. 11.—Prophase II, showing the "saucer" stage, *A. cordifolia*. 12.—Metaphase II, *A. cordifolia*. 13-20.—*Backhousia citriodora*. 13.—Prophase I. 14.—Diakinesis, showing the chromosomes arranged at the surface of the nucleus. 15.—Metaphase I. The cell has been pressed from its enclosing wall and the chromosomes are all sharply focused. 16.—Metaphase I showing secondary pairing. 17.—Anaphase I slightly in oblique view. 18.—Interphase nuclei in early prophase II. 19.—Metaphase II. 20.—Anaphase II. The two spindles here lie in the same plane.

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